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Short Communication

A rapid and sensitive determination of paclitaxel in rat plasma by UPLC-MS/MS method: Application to a pharmacokinetic study

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ABSTRACT

A rapid and sensitive method for quantitative determination of paclitaxel in rat plasma was developed and validated by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Docetaxel was used as an internal standard and diethyl ether was the liquid–liquid extraction agent. Multiple reaction monitoring (MRM) mode via positive electrospray ionization (ESI) was applied to detect paclitaxel and IS at the transitions *m/z* 854 → 286 and *m/z* 808.48 → 527.3, respectively. This method covered a linearity range from 5 to 5000 ng/ml, with the total run time of 3.0 min. In summary, a high-throughout UPLC-MS/MS method was successfully developed to measure paclitaxel in rat plasma and was applied to pharmacokinetic study after intravenous administration of paclitaxel.

1. Introduction

Paclitaxel (PTX) is a natural hydrophobic diterpenoid extraction from the bark of the Pacific yew. As mitotic inhibitor, paclitaxel can stabilize and protect microtubules from disassembly, and then interfere with the normal breakdown of microtubules during cell proliferation. As an effective cancer chemotherapy agent, it has been widely used for the treatment of various tumors, including ovarian, breast, non-small cell lung, and prostate tumors [1–3]. Therefore, its commercial formulation (Taxol®, Bristol-Myers Squibb, New York, NY) has been comprehensively used in clinic treatment [4–6]. For example, it has been used for the first-line and second-line treatment of ovarian cancer and nonsmall cell lung cancer which is unsuitable for curative treatment. To achieve better chemotherapy efficacy, it is necessary to timely monitor the pharmaceutical pharmacokinetic parameters after intravenous administration.

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Analytical methods for determination of paclitaxel in plasma have already been reported. Earlier methods concentrated on the high performance liquid chromatography (HPLC) with UV detector [7–9]. However, its application has been severely limited due to its weakness of sensitivity and long run time. Later, with the development of MS detector, liquid chromatography-mass spectrometry (LC-MS) method [10,11] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are widely used for determination of paclitaxel in biological samples. Vainchtein LD et al. [12] used an LC-MS/MS method to determine plasma concentration of paclitaxel with an LLOQ of 0.25 ng/ml, but the chromatographic separation time was longer than 6 min and the plasma spiked volume was 200 μl. With the introduction of ultra-performance liquid chromatography (UPLC), the analysis efficiency has been apparently improved. Zhang SQ et al. [13] reported by using UPLC-MS/MS method, the chromatographic separation time was achieved within 2 min with an LLOQ of 5 ng/ml, but this method was only used for the determination of paclitaxel in human plasma.

The aim of this research was to establish and validate a rapid and sensitive method for quantitatively determination the rat plasma concentration of paclitaxel using UPLC-MS/MS technique. The validation results showed this method was accurate, sensitive, high-throughout and suitable for application in pharmacokinetic studies.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX, 99.9% purity) and docetaxel (DTX, internal standard, I.S., 99.9% purity) were obtained from Xi'an Helin Biological Engineering Co. Ltd. (Xi'an, China). Commercial Taxol solution was obtained from Shanghai Quanyu Pharm. Co. (Shanhai, China). Acetonitrile and methanol of HPLC-grade were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ultrapure water (>18 MΩ) was prepared by Easypure II RF/UV system (Boston, MA, USA). Formic acid of HPLC-grade was purchased from Dikma (Richmond Hill, NY, USA). Diethyl ether of analytical grade was purchased from Concord Tech. Co. (Tianjin, China) and high-purity nitrogen (99.999%) was used. The plasma sample was collected at designated time from the ocular region of the rats after centrifugation for 10 min. All the other reagents were of analytical grade.

2.2. UPLC-MS/MS conditions

Analyses were carried on an ACQUITY TQD system (Waters Corp., Milford, MA, USA) equipped with cooling autosampler, column oven and an ACQUITY triple-quadrupole tandem mass spectrometric detection with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA). An ACQUITY UPLCTM BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters Corp, Milford, MA, USA) was used. A gradient elution was used to achieve chromatographic separation with a mobile phase composed of mobile phase A (acetonitrile) and mobile phase B (water, containing 0.1% formic acid). The mobile phase in the gradient elution progress was: 0 min 50% B, 1.6 min 35% B, 2.5 min 50% B and 3 min 50% B. The flow rate was set at 0.2 ml/min and the column temperature was maintained at 40 °C. The auto-sample temperature was set at 4 °C and the injection volume was 10 μl using the partial loop mode for sample injection. The analytical run time for each sample was 3.0 min. From 1.0 to 2.5 min, the elution was injected into the detector, and the remainder was diverted to waste.

The separated compounds were detected by a Water Tandem Quadrupole (TQ) Detector (Waters). Paclitaxel and I.S. were monitored with the mass spectrometer by positive electrospray ionization (ESI) interface mode. The ionization source conditions were: source temperature 120 °C, capillary voltage 3.13 kV, cone voltage 35 V and desolvation temperature 380 °C. The cone and desolvation gas flow rates were 50 and 550 l/h, respectively. The optimal collision energy was 20 and 10 V for paclitaxel and I.S., respectively. Argon was used as collision gas at a pressure of approximately 0.0033 mbar. Samples were analyzed by multiple reaction monitoring (MRM) of the transitions of *m/z* 854 → 286 for PTX and *m/z* 808.48 → 527.3 for I.S., respectively. The scan time was set at 0.2 s per transition. Data were acquired by Masslynx 4.1 software.

2.3. Preparation of standards and quality control samples

Stock solutions of paclitaxel and I.S. were prepared in acetonitrile at a concentration of 100 μg/ml, respectively. To prepare the working standard PTX solutions of desired concentrations, the PTX stock solution was serially diluted with mobile phase composed of solvent A (acetonitrile, 50%) and solvent B (water, containing 0.1% formic acid, 50%). The

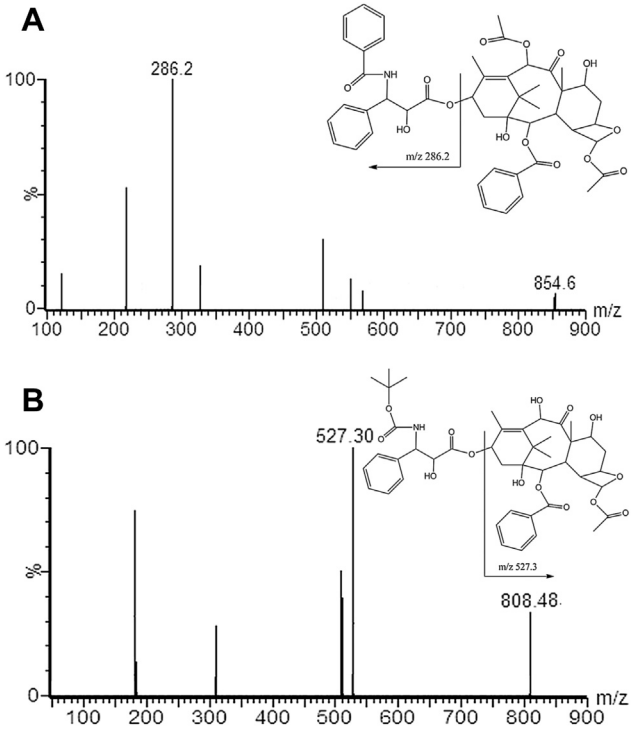

Fig. 1 – Product ion scan spectra of paclitaxel (A) and docetaxel (B).

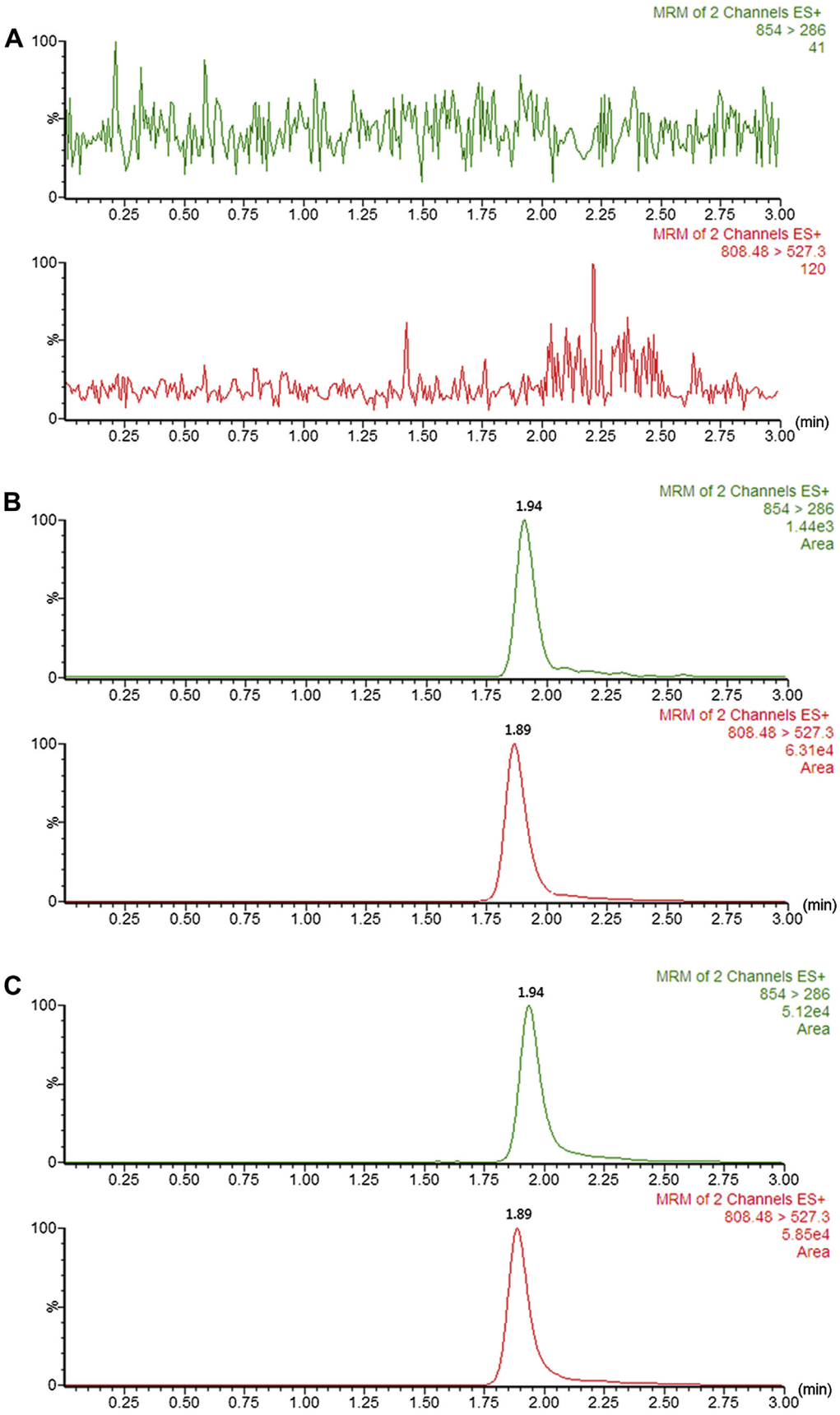

Fig. 2 – Representative MRM chromatograms of paclitaxel and docetaxel in rat plasma samples. (A) Blank plasma sample. (B) The blank plasma sample spiked with paclitaxel at an LLOQ of 5 ng/mL and IS. (C) Plasma sample from a rat, 10 min after intravenous administration of Taxol solution. The retention time for paclitaxel and docetaxel was 1.94 and 1.89 min, respectively.

I.S. working solution was diluted with the mobile phase to a final concentration of 1 μg/ml.

Calibration standards were prepared by spiking 50 μl drug-free rat plasma with 50 μl of appropriate PTX standard solutions. The effective concentrations in plasma samples were 5, 10, 50, 100, 200, 500, 1000, 2000 and 5000 ng/ml. The quality control samples were prepared in a similar way to the calibration standards at concentrations of 10, 200 and 4000 ng/ml.

2.4. Sample preparation

To a 50 μl of rat plasma, 50 μl of mobile phase and 50 μl of I.S. solution (DTX, 1 μg/ml in the mobile phase) were added. Then the sample mixture was vortexed for 1 min. To extract the sample, 3 ml diethyl ether was added and then vortexed for 3 min, followed by centrifuged at 3500 rpm for 10 min. Then the upper organic layer was taken out and evaporated to dryness under a gentle nitrogen stream at 37 °C. The residue was reconstituted by 100 μl of mobile phase. After centrifuged at 13,000× *g* for 10 min, a 10 μl aliquot of the supernatant was then injected into the UPLC-MS/MS system for assay.

2.5. Method validation

The analytical method for quantitative determination of PTX in rat sample was validated for selectivity, carryover, lower limit of quantification (LLOQ), linearity, accuracy, precision, matrix effect, extraction recovery, and stability. The different validation parameters and the values for accepting the range of validation parameters were in accordance with international guidelines [14] and Food and Drug Administration guidelines (www.fda.gov/cvm).

Selectivity was investigated by comparing chromatograms of six different batches of blank plasma obtained from six rats with those of corresponding standard blood samples spiked with PTX and I.S. and a plasma sample obtained after intravenous administration.

Carryover was estimated by six continual injections of an extracted blank sample after the injection of an extracted upper limit of quantitation sample.

Quantitative determination of the plasma samples was based on the ratio of the detector response of paclitaxel to IS. The standard curves in the form of $y = A + BX$ were calculated by plotting the peak area ratio (Y) against paclitaxel concentration (X) using weighted ($1/x^2$) least squares linear regression. During routine analysis, each analytical run was consisted of a set of calibration standards, a set of QC blood samples in duplicate at intervals per batch and the blood samples to be analyzed.

Nine standard plasma samples ranging from 5 to 5000 ng/ml were used to prepare the calibration standards. LLOQ was defined as the lowest concentration of paclitaxel on the calibration curve. The precision and accuracy were assessed by analyzing six samples which were prepared in six replicates, and the result should be less than 20%. Meanwhile, the signal/noise ratio should be over 10.

The accuracy, intra- and inter-day precision were assessed using QC samples at three concentration levels (10, 200 and 4000 ng/ml) on three separate days. The accuracy was defined as the relative error (R.E.) and the precision was determined as the relative standard deviation (RSD).

To investigate the matrix effect (ME), six different lots of blank plasma were extracted and the spiked with the analyte at different paclitaxel concentrations (10, 200 and 4000 ng/ml). The corresponding peak areas (*A*) were then compared with those of the paclitaxel standard solutions in mobile phase (*B*). There was a matrix effect when the ratio ($A/B \times 100$) % was <85% or >115%. The matrix effect of I.S. was evaluated in the same procedure.

Extraction recovery of paclitaxel was defined as the ratio between the mean peak area of the regularly extracted samples (10, 200 and 4000 ng/ml) and the mean peak area of spike-after-extraction plasma samples. The extraction recovery of IS was evaluated in the same way using the medium level of QC as a reference.

The stability of this method including sample post-treatment (in the reconstituted extract at room temperature for 4 h), sample storage (at room temperature for 24 h) and freeze-thaw stability (three cycles) was investigated by analyzing replicates ($n = 3$) of QC samples (10, 200 and 4000 ng/ml). The results were compared with those freshly prepared QC samples and the percentage of concentration deviation was calculated.

2.6. Pharmacokinetic study

The developed method was used to determine the plasma concentration of paclitaxel in Taxol injection after intravenous administration in six healthy adult female SD rats weighing 220 ± 20 g (Laboratory Animal Center of Shenyang Pharmaceutical University, Shenyang, Liaoning, China). The rats were deprived of food overnight but with free access to water before experiments. All animals investigated in this research were executed according to the Guidelines for the Care and Use of Laboratory Animals approved by the Ethics Committee of Animal Experimentation of Shenyang Pharmaceutical University.

The Taxol solution was diluted with 0.9% NaCl to produce a solution with PTX concentration of 1.5 mg/ml. The rats were treated with diluted Taxol solution at dose of 8 mg/kg by intravenous administration. Blood samples were collected into heparinized tubes at 0.0833, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8 and 10 h and centrifuged immediately at 13,000 g for 10 min. Then the plasma was frozen at −80 °C for analysis.

3. Results and discussions

3.1. Method development

Paclitaxel has a higher sensitivity achieved by ESI source rather than APCI and a stronger response in the positive-ion

Table 1 – Accuracy and precision for paclitaxel at the plasma concentration of LLOQ (six replicates).

Analytes	Added concentration (ng/mL)	Found concentration (ng/mL)	SD (ng/mL)	RSD (%)	RE (%)
Paclitaxel	5	5.398	0.317	5.9	8.0

Table 2 – Precision and accuracy of the UPLC-MS/MS method to determine paclitaxel in rats plasma ($n = 3$ days, six replicates per day).

	Added conc. (ng/mL)	Found conc. (ng/mL)	R.S.D. (%)	R.E. (%)
Day 1	10	9.80	9.64	−2.00
	200	176.47	1.38	−11.77
	4000	3579.89	3.87	−10.50
Day 2	10	10.60	3.88	5.98
	200	175.34	1.68	−12.33
	4000	3607.87	3.24	−9.80
Day 3	10	9.99	8.14	−0.07
	200	176.79	1.36	−11.60
	4000	3746.77	2.04	−6.33

mode compared with the negative-ion mode. So ESI^+ mode was used for the detection of paclitaxel and IS. Through positive ion full-scan spectra, the predominant protonated molecular ion $[M + H]^+$ for the analytes were 854 and 808.48, respectively. In order to capture the strongest ion intensity, parameters such as ESI source temperature, capillary and cone voltage were optimized. Then in the collision induced dissociation condition, the abundant product irons were obtained at *m/z* of 286 and 527.3 for paclitaxel and IS, respectively. The product-ion spectra of these compounds are shown in Fig. 1.

In the chromatographic optimizing process, Acetonitrile was used in the mobile phase instead of methanol because of its lower background noise. Adding formic acid could obviously improve detection sensitivity presumably induced by facilitative ionization of the analytes. Therefore, the mobile phase was consisted of water (0.1% formic acid)-acetonitrile. As described in Section 2.2, a gradient elution progress was used to completely separate paclitaxel and IS. The total run time for both the analytes was 3.0 min. From 1.0 to 2.5 min, the elution was injected into the detector, and the remainder was diverted to waste for avoidance the contamination of ion source by impurities.

Protein precipitation and liquid–liquid extraction methods were compared during the sample preparation. The former method was discarded because of high noise and the poor LLOQ of only 25 ng/ml. In the liquid–liquid extraction method, diethyl ether was used as the extraction solvent, the higher extraction and lower background noise were also obtained. Nevertheless, the extraction recovery was only about 70% when the samples were extracted by 2 ml diethyl ether, so we added 3 ml diethyl ether to improve the extraction recovery. The following steps were conducted as Section 2.4.

3.2. *Method validation*

3.2.1. *Selectivity*

As shown in Fig. 2, it was obviously that there was no interference from endogenous substances in blood at the retention time of the analytes.

3.2.2. *Carryover*

Carryover study indicated that the carryover from residues in sampling/switching valves and late-eluting residues in the column was both negligible.

3.2.3. *Linearity and LLOQ*

The linear regression was obtained between the peak area ratios and the paclitaxel concentration ranging from 5 to 5000 ng/ml in rat plasma. A typical calibration curve equation was:$y = 2.18903 * x + 15.2838 (r = 0.9960)$, where y is the peak area ratio of PTX to IS, and *x* is the concentration of PTX. This concentration range showed a good linearity ($r > 0.99$) in all analytical runs. The calibration curve for each run was exhibited in Table 4.

LLOQ was established by this method at concentration of 5 ng/ml for paclitaxel, which was suitable for quantitative determine the plasma concentration of Taxol injection after intravenous administration. The accuracy and precision values of LLOQ were exhibited in Table 1.

3.2.4. *Precision and accuracy*

Table 2 summarized the accuracy and precision for paclitaxel from QC samples. The data was listed for each day individually. The results showed a good precision that all the values were below 9.64%. The accuracy ranged from −12.33% to 5.98%, which was also acceptable. The results above illustrated that this UPLC-MS/MS method was precise and accurate.

3.2.5. *Matrix effect*

All the matrix effect ratios ($A/B \times 100$) % for PTX and I.S. were between 91.73% and 103.39%, which were within the acceptable limits (90.0%–110.0%). This result means that there was no ion suppression and enhancement phenomenon from plasma matrix.

Table 3 – Stability of paclitaxel in rat plasma (three replicates).

Storage conditions	Added conc. (ng/mL)	Found conc. (ng/mL)	R.S.D. (%)	R.E. (%)
24 h at room temperature	10	9.85	5.34	−1.53
	200	190.02	0.97	−4.99
	4000	4140.42	1.78	3.51
4 h at room temperature after extraction	10	10.47	2.69	4.70
	200	193.51	3.09	−3.24
	4000	3854.14	2.20	−3.65
After three freeze/thaw cycles	10	10.25	2.64	2.50
	200	190.44	2.90	−4.78
	4000	4069.64	1.99	1.74

Table 4 – The calibration curve of paclitaxel for each run.

Run	Calibration curve	r
1	$y = 2.18903 * x + 15.2838$	0.9960
2	$y = 1.5711 * x + 11.2554$	0.9923
3	$y = 2.43747 * x + 26.2739$	0.9969
4	$y = 1.64999 * x + 32.4629$	0.9936
5	$y = 1.13436 * x + 7.98519$	0.9975
6	$y = 2.21728 * x + 14.958$	0.9917

3.2.6. *Extraction recovery and stability*

Mean extraction recoveries of PTX at 10, 200, 4000 ng/ml were 90.15 ± 3.51%, 98.47 ± 7.77% and 88.30 ± 2.23%, respectively. The extraction recovery of I.S. was 90.31 ± 4.67%.

The relative errors of all the QC samples in the stability experiment were below 5.5%, which could be seen in Table 3, indicating that there was no significant degradation of PTX under the condition described previously.

3.3. *Application*

This developed method was successfully used to determine the plasma concentration of paclitaxel after intravenous administration of Taxol injection (8 mg/kg). At designated times, blood sample were collected up to 10 h after intravenous administration. Our preliminary work indicated that the high PTX blood concentrations of the first 30 min samples were out of the calibration range, so these samples were detected after 10 fold dilution. The feasibility of this method was also validated in our previous work (Data not shown).

The mean plasma concentration–time curve of paclitaxel is shown in Fig. 3. The results showed that after single dose of 8 mg/kg Taxol solution, the mean half-life ($T_{1/2}$) and the mean residence time (MRT) were calculated to be 5.85 ± 3.42 h and 2.91 ± 1.07, respectively. The mean area under the plasma concentration versus time curve ($AUC_{0-\infty}$) was 15.05 ± 3.12 μg h/ml.

Regarding the relatively easy sample preparation method (liquid–liquid extraction) and short chromatographic run time (within 3 min), about 200 samples could be analyzed routinely per day. This convenient and rapid method is able to meet the requirements of pharmacokinetic study for paclitaxel.

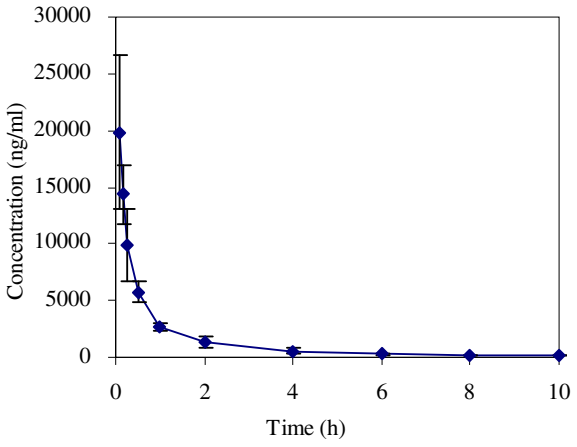

Fig. 3 – Mean plasma concentration–time curve of paclitaxel after a single intravenous dose of 8 mg/kg to six rats (mean ± SD).

4. Conclusion

We have developed and validated a rapid and sensitive UPLC-MS/MS method for quantitatively high-throughput determination of paclitaxel. Through a rapid liquid–liquid extraction process, this method was suitable for application in pharmacokinetic studies of paclitaxel in rats after intravenous administration.

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REFERENCES

[1] Kingston DG. The chemistry of taxol. Pharmacol Ther 1991;52:1–34.
[2] Arbuck SG, Christian MC, Fisherman JS, et al. Clinical development of taxol. J Natl Cancer Inst Monographs 1993;15:11–24.
[3] Rowinsky EK, Onetto N, Canetta RM, et al. Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 1992;19:646–662.
[4] Hogberg T, Glimelius B, Nygren P. A systematic overview of chemotherapy effects in ovarian cancer. Acta Oncol 2001;40:340–360.
[5] Garces AH, Mora PA, Alves FV, et al. First-line paclitaxel and carboplatin in persistent/recurrent or advanced cervical cancer: a retrospective analysis of patients treated at Brazilian National cancer Institute. Int J Gynecol Cancer 2013;23:743–748.
[6] Del Mastro L, Fabi A, Mansutti M, et al. Randomised phase 3 open-label trial of first-line treatment with gemcitabine in association with docetaxel or paclitaxel in women with metastatic breast cancer: a comparison of different schedules and treatments. BMC Cancer 2013;13:164.
[7] Kim SC, Yu J, Lee JW, et al. Sensitive HPLC method for quantitation of paclitaxel (Genexol in biological samples with application to preclinical pharmacokinetics and biodistribution. J Pharm Biomed Anal 2005;39:170–176.
[8] Wang LZ, Ho PC, Lee HS, et al. Quantitation of paclitaxel in micro-sample rat plasma by a sensitive reversed-phase HPLC assay. J Pharm Biomed Anal 2003;31:283–289.
[9] Lee SH, Yoo SD, Lee KH. Rapid and sensitive determination of paclitaxel in mouse plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1999;724:357–363.
[10] Parise RA, Ramanathan RK, Zamboni WC, et al. Sensitive liquid chromatography-mass spectrometry assay for quantitation of docetaxel and paclitaxel in human plasma. J

Chromatogr B Analyt Technol Biomed Life Sci 2003;783:231–236.
[11] Guo P, Ma J, Li S, et al. Determination of paclitaxel in mouse plasma and brain tissue by liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003;798:79–86.
[12] Vainchtein LD, Thijssen B, Stokvis E, et al. A simple and sensitive assay for the quantitative analysis of paclitaxel and metabolites in human plasma using liquid chromatography/tandem mass spectrometry. Biomed Chromatogr 2006;20:139–148.
[13] Zhang SQ, Chen GH. Determination of paclitaxel in human plasma by UPLC-MS-MS. J Chromatogr Sci 2008;46:220–224.
[14] Shah VP, Midha KK, Findlay JW, et al. Bioanalytical method validation – a revisit with a decade of progress. Pharm Res 2000;17:1551–1557.